**To:** BLA STN 103780
**From:** Gary Kikuchi
**Through:** Elizabeth Shores, DTP, Amy Rosenberg, Director DTP
**Date:** March 6, 2002

**Immunogenicity Review for Serono Rebif BLA**

## I. Administrative:

Immunogenicity Reviewer: Gary Kikuchi
Product Reviewer/BLA chair: Gibbes Johnson
Pharm/Tox reviewers: Anne Pilaro, Dave Green
Clinical Review team: Cynthia Rask, Ellis Unger, Marc Walton (branch chief)
Statistical Reviewer: Clare Gnecco
RPMs Susan Guiliani, Karen Winestock

**Milestones:** Action due date March 7, 2002

## II. Summary

### A. Product

Rebif is interferon-beta manufactured by Serono for treatment of multiple sclerosis. The following table summarizes the status of commercial preparations of interferon-beta. In the current BLA, Serono is seeking US licensing for Rebif.

| Company | Trade Name | Generic name | Host cell | 1o sequence | Glycos-ylated | Licensed route | US license |
|---|---|---|---|---|---|---|---|
| Biogen | Avonex | IFN-beta-1a | CHO | Native | Yes | IM | Approved |
| Berlex/Chiron | Betaseron | IFN-beta-1b | E.Coli | Ser17Cys | No | Sub Q | Approved |
| Serono | Rebif | IFN-beta-1a | CHO | Native | Yes | Sub Q | Submitted |

### B. Clinical trials examining antibody to interferon beta

The sponsor has submitted several clinical trials to support licensure of this product. The PRISMS trial (XXXXXXXXXX) studied 560 patients with relapsing-remitting MS (RRMS) in a multicenter, randomized, placebo-controlled trial that compared either 22 mcg or 44 mcg of Rebif vs. placebo 3x/week subQ for 2 years. More recently, the EVIDENCE trial (XXXXXXXXXX) enrolled up to 624 patients with RRMS in an open-label randomized, multicenter trial comparing Rebif 44 mcg 3x/week subQ versus Avonex 30 mcg 1x/week IM for 48 weeks.

Clinical immunogenicity data from the PRISMS (XXXXXXXXXX) trial are used to support labeling. Summary immunogenicity data were provided Jan 11, 2002 and line listings supporting the summary data were provided February 6, 2002 and have been analyzed for consistency with the summary data. Because the clinical cutoff value of

XXXXXXXXXX in the neutralization assay was not validated, the incidence rates were calculated based on patients with any positive neutralizing titer to Rebif. In patients treated with the approved 44 mcg dose of Rebif, 45 patients had positive neutralizing titer, and the total number of patients was 184, so the incidence rate (calculated on an intent-to-treat basis) was 45/184 (24%).

**C. Summary of issues with immunogenicity assays**

The ELISA screening assay is appropriately validated for cutoff, sensitivity, specificity, and robustness. However, additional information is needed regarding assay validation, including:

- Assay precision using human positive control antiserum, and information regarding the source of this antiserum.
- Assurance that the ELISA screening assay has equivalent activity for antibodies to all forms of interferon beta used in the EVIDENCE (XXXXXXXXXX) trial.

The neutralizing antibody assay is appropriate in terms of design and validation of assay sensitivity with respect to WHO international standards. Because of this, the incidence rate from the PRISMS (XXXXXXXXXX) study can be used in labeling. However, additional information is needed regarding certain elements of assay validation, including:

- Assay intermediate precision, including assurance of uniformity among operators
- Calculation of descriptive statistics, including mean, standard deviation, and % CV on the log transform of neutralization titers
- Assay sensitivity, with particular regard to validation that the assay limit of quantification is lower than the cutoff value used to analyze clinical data
- Assay specificity, including data that support the statement that the assay can distinguish between antibodies to interferon alpha and interferon beta
- Assurance that the neutralizing antibody assay has equivalent activity for antibodies to all forms of interferon beta used in the EVIDENCE (XXXXXXXXXX) trial
- Assay reproducibility between sites, including assurances that the assay precision is acceptable and equivalent at both sites

Phase IV commitments have been provided by the sponsor to address these issues (see below).

## III. ELISA screening assay for immunogenicity

### A. Description of assay methods

1. Overview

The ELISA screening assay, described in documents XXXXXXXXXX, was developed at Serono Diagnostics (XXXXXXXXXX), and subsequently transferred to XXXXXXXXXX for routine use. In the ELISA design, XXXXXXXXXX.

2. Detailed methods (from SOP CLI048V4)
XXXXXXXXXX

3. Definitions and calculations

XXXXXXXXXX is used to perform the calculations on the raw data. A cutoff value of mean XXXXXXXXXX(see below) is calculated by the program. Results less than this cutoff are negative. Any positive samples are absorbed with Rebif and re-screened, as indicated above.

In some of the validation documents, ELISA results are expressed as the ratio of the observed value to the negative control. As stated by the sponsor in the telecon of February 28, 2002, this analysis is performed to reduce inter-assay variability due to different sources of normal human serum.

### B. Assay Cutoff Value.

**1.** Method and Definition. 34 samples from normal human controls were analyzed in the ELISA and the mean and standard deviation of these controls were calculated. In the experiment validating assay limit of detection, the mean OD of normal human serum was XXXXXXXXXX. The cutoff value was defined as mean XXXXXXXXXX times the standard deviation, which is equivalent to XXXXXXXXXX times the mean.

**2.** Outcome. The definition of the ELISA cutoff in terms of mean XXXXXXXXXX is appropriate.

## IV. ELISA Assay Validation

### A. Assay Precision.

1. XXXXXXXXXX

2. Outcome. Additional validation of assay precision is needed. The first study described above does not use human serum and therefore does not completely

validate the secondary detection reagent used. The second study does not use human serum as a positive control. The source of the human positive control serum should be identified.

**B. Assay Sensitivity**
**XXXXXXXXXX**
**Assay Linearity**
**XXXXXXXXXX**
**C.Assay Specificity**
**XXXXXXXXXX**

D.Assay Robustness

XXXXXXXXXX

Outcome. The ELISA SOP was optimal with respect to the above variables.

B. Assay Reproducibility

Formal studies demonstrating reproducibility of technology transfer from the XXXXXXXXXX site to the XXXXXXXXXX site were not provided in the validation information. However, validation studies from both sites are described above. In particular, assay precision, assay sensitivity, and assay specificity were validated at the XXXXXXXXXX site.

**V. Neutralizing antibody assay for immunogenicity**

**A. Description of assay methods**

1. Overview:
   The Serono neutralizing antibody assay was developed at XXXXXXXXXX.

2. Detailed Methods:
XXXXXXXXXX

3. Definitions and calculations:

The titer of neutralizing antibodies is expressed in neutralizing units/ml by the formula

XXXXXXXXXX.

The definition of lab units (LU) is described above.

4. Problems/Caveat/Comments:
   a. Data Calculations.
      The sponsors use the formula
      XXXXXXXXXX

Grossberg SE and Kawade Y (Biotherapy 1997, 10: 93) currently recommend a slightly different formula for data calculations, which is:

NU/ml = observed titer x (LU/ml – 1)/9

Although the formula used by the sponsors is not the one recommended by Grossberg, it has been retained for consistency with historical assays previously performed. In addition, the difference between the two formulas is small at the concentration of interferon used (XXXXXXXXXX)

b. There are XXXXXXXXXX, which means that there are XXXXXXXXXX in each well.

B. Assay cutoff value

The assay cutoff value has not been determined. The assay cutoff value means the titer, expressed in neutralizing units, which must be achieved to consider a sample positive. The sponsor analyzes the clinical incidence rates in terms of titers XXXXXXXXXX, implying that the cutoff value is XXXXXXXXXX. A cutoff value of 20 is mentioned in the literature for neutralizing antibody assays for interferon beta in general (Pungor E. et al., J. Interferon Cytokine Res. 1998, 18: 1025.) However, in that reference, the assay is for neutralizing antibodies to interferon beta-1b rather than antibodies to interferon beta-1a. The clinical cutoff value chosen must be validated to be greater than the limit of quantitation of the assay. Because the cutoff value has not been validated, all positive titers greater than zero are used to calculate the incidence rate.

## VI. Neutralizing Assay Validation

### A. Assay precision

1. Method

To support assay precision, XXXXXXXXXX. The arithmetic mean of the titer, and corresponding standard deviation and %CV were calculated by the sponsor and are shown below. The table also shows the mean of the log 10 titer, standard deviation, and %CV as calculated by this reviewer. Use of log 10 titers is mentioned in Siber GR and Ransil BJ, Methods Enzymol. 1983, 93: 60.

XXXXXXXXXX

2. Outcome. Validation of assay precision is deficient.

3. Problems/Caveats/Comment
   a. Only one experiment is provided to support precision validation, and it was performed at the XXXXXXXXXX.

b. The assay validation is deficient, as intermediate precision using different operators was not assessed.

**B. Assay sensitivity**

1. Method.

XXXXXXXXXX

From the raw data provided by the sponsor, the log 10 titer, mean log titer, standard deviation of the log titer, and % CV were calculated by this reviewer XXXXXXXXXX.

XXXXXXXXXX.

2. Outcome. Validation of the sensitivity of the assay is appropriate, because the value for the WHO antiserum is correct.

3. Problems/Caveats/Comments
   a. Although the titer for the WHO antiserum is correct, the assay sensitivity is not validated in terms of the limit of detection and limit of quantification.
   b. The assay is not validated to detect antibodies to Avonex, which was used in the XXXXXXXXXX clinical trial, and assay sensitivity has not been validated using Avonex as an antigen.

**C. Assay Linearity**

1. XXXXXXXXXX.
2. XXXXXXXXXX

3. Problems/Caveats/Comments

According to ICH Q2B, the linearity of the assay should be addressed over the entire range of the assay using at least five data points. This assessment of linearity is deficient.

An additional problem that relates to linearity is that the sponsor performs descriptive statistics (calculation of mean, standard deviation, and %CV) on the titers rather than the log transformed titers. When the titer is very large, the standard deviation and the %CV are extremely large and are not meaningful. Use of log transformations of titers was described by Siber and Ransil (Methods Enzymol 1983, 93: 60) In order to facilitate comparisons, this reviewer has re-calculated log titers and the mean, standard deviation, and %CV on the log titers. The sponsor should perform this analysis. This type of analysis falls under validation of assay linearity in the ICH Q2A and Q2B.

**D. Assay specificity**

1. Method

In the information provided to support validation, the sponsor also tested the titer of WHO international standard antibodies to interferon alpha using various interferon alpha preparations.

2. Outcome: The sponsor stated that the WHO international standard interferon beta serum did not cross-react with the interferon alpha preparations and vice versa.

3. Problems/Caveats/Comments
   a. The sponsor did not show any data supporting this statement.

**E. Robustness (optimization)**

1. Method of determination: Not done. Robustness of the assay, or whether the assay is optimized for all important parameters including interferon beta concentration, and serum freeze-thaw, should be performed prior to establishment of the assay, and these data should be provided to support validation.
2. Outcome: Assay robustness was not addressed for the neutralization assay.

Although this information was provided for the ELISA, information regarding assay robustness is performed during development and is not covered under ICH Q2A/Q2B.

**F. Assay reproducibility (technology transfer between sites)**

1. Method:
   The neutralizing antibody assay was developed at the XXXXXXXXXX site and transferred to the XXXXXXXXXX site. To validate assay reproducibility, or the technology transfer between sites, the following two experiments were performed:
   a. XXXXXXXXXX
   b. XXXXXXXXXX
2. Outcome. At both sites, the WHO antiserum was measured correctly within experimental error.
3. Problems/Caveat/Comment
   There is much greater %CV at the XXXXXXXXXX site in both experiments. This appears to indicate that measurement of the titer at XXXXXXXXXX is not as precise as at XXXXXXXXXX, indicating a problem with assay reproducibility.

**V. CBER approved labeling.**

As with all therapeutic proteins, there is a potential for immunogenicity. The presence of neutralizing antibodies (NAb) to Rebif® (INF beta-1a) was determined by collecting and analyzing serum pre-study and at 6 month time intervals during the 2 years of the clinical trial. Serum NAb were detected in 45/184 (24%) of patients at one or more times during the study in the Rebif 44 mcg group. The clinical and pathological significance of the presence of NAb is unknown.

The data reflect the percentage of patients whose test results were considered positive for antibodies to Rebif® (interferon beta-1a) using an antiviral cytopathic effect assay, and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of NAb positivity in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to Rebif® with the incidence of antibodies to other products may be misleading.

**Proposed post-marketing commitments for Serono Rebif:**

In order to address the issues with the immunogenicity assays, the following post-marketing commitments discussed with the sponsor on March 4, 2002 and were formally proposed by the sponsor in their draft document dated March 5, 2002:

1. Additional validation for the ELISA:

    a. XXXXXXXXXX
    b. Demonstration that the ELISA screening assay has equivalent activity for antibodies to all forms of interferon beta used in clinical study XXXXXXXXXX.

**V. Additional validation of the neutralizing antibody assay, according to the guidance in XXXXXXXXXX with respect to:**

a. XXXXXXXXXX
b. XXXXXXXXXX.

c. Assay specificity. Data supporting the statement that the assay can distinguish between antibodies to interferon alpha and interferon beta.

d. XXXXXXXXXX.

e. XXXXXXXXXX.

Most of the validation data identified above will be submitted to the BLA file concurrently with additional data from study XXXXXXXXXX, in June 2002. Data to address item 2c above (assay specificity) will be submitted in September 2002.